

Byssochlamys nivea Growth in Papaya Juice as Influenced by Water Activity and Ascospore Age

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Abstract

In a previous study on *Byssochlamys nivea* growth in pineapple juice (Zimmermann et al., 2011a), the water activity (a_w) was shown to be statistically significant ($p < 0.05$) on growth parameters, while Ascospore Age (AA) did not influence the microorganism growth. Since the study of thermal resistant moulds is relevant to the industry and the medium composition has an important effect on the growth/inactivation of microorganisms, the evaluation of a_w (0.90 to 0.99) and AA (30 to 90 days) influence on growth parameters of *B. nivea* in a different juice, papaya juice, is the main purpose of this study. Primary models were fitted to experimental data. The results showed that AA did not influence mould growth; nevertheless, a_w was statistically significant for the parameters λ and μ_{max} . These results confirm the previous study, concluding that the growth parameters of *B. nivea* were influenced by a_w , irrespective of the kind of juice. Secondary models described the influence of the significant factor on the growth parameters. In an attempt to contribute to the food industry, the present study evaluated the growth of *Byssochlamys nivea* in papaya juice as influenced by water activity and ascospore age. The findings will be useful when developing strategies to prevent growth of *B. nivea* in acid fruit juice.

Keywords

Byssochlamys nivea; Growth Modeling; Water Activity; Ascospore Age; Tropical Fruit Juice

Introduction

Byssochlamys species are often associated with fresh fruits since these microorganisms are into soil and the products may be contaminated by contact with the soil or rain splash. *Byssochlamys* sp. produces extremely resistant ascospores that are responsible for spoilage of acid processed foods such as fruit juices and fruit based products (Chapman et al., 2007). The sexual

reproduction of *Byssochlamys nivea* is provided by ascospores which are produced in saclike specialized terminal cells called asci (Beuchat and Rice, 1979). They can grow at low oxygen tension and in acid environments (Taniwaki et al., 2009); and resist commercial heat treatments normally applied to pasteurized fruit juices and fruit products (Tournas and Traxler, 1994).

Fungal species that produce ascospores commonly reported in fruit based products are: *Byssochlamys fulva* and *B. nivea*, *Neosartorya fischeri*, *Talaromyces* sp. and *Eupenicillium* sp. (Pitt and Hocking, 1999). Besides being a heat-resistant mould, *B. nivea* can be considered a potential producer of the mycotoxin patulin in pasteurized products (Sant'Ana et al., 2008; Sant'Ana et al., 2010b). Patulin is a toxic metabolite that can cause acute symptoms, including nervousness, convulsions, lung congestion, edema, hyperemia, gastrointestinal tract distension, intestinal hemorrhage and epithelial cell degeneration (Mahfoud et al., 2002).

Environmental factors such as oxygen tension, soluble solids, organic acids, pH, water activity and temperature significantly affect mould growth (Panagou et al., 2010). So far, limited studies have been published on the effect of those environmental factors on the growth of heat-resistant mould (Panagou et al., 2010). Valík and Piecková (2001) investigated the effect of a_w (0.850 to 0.995) on the growth responses of *Byssochlamys fulva*, *Neosartorya fischeri* and *Talaromyces avellaneus* at 25 °C, and they found that the germination and growth of heat resistant mould can be reduced by lowering the water activity. Roland and Beuchat (1984) stated that the minimum level of a_w that allowed *B. nivea* to grow was 0.89 when the

temperature was at 30 °C. Also, Panagou et al. (2010) reported the minimum a_w level for *B. nivea* growth was 0.89 and the best growth temperature was at 32 °C.

A large number of researches have shown the presence of organic acids in the medium highly affect growth/inactivation of microorganisms. Salomão et al. (2007) found that citric acid from pineapple and papaya juices had a greater influence on the thermal resistance of ascospores of *Neosartorya fischeri* than malic acid from apple juice. Rajasshekhara et al. (2000) studied the effect of organic acids on the thermal death of *Neosartorya fischeri* in mango juice at 85 °C and reported that tartaric acid took almost 3 times longer to reach 3 log reductions of the mould than malic, citric and lactic acids. Conversely, some works did not find differences on growth/inactivation of microorganisms among media supplemented with different organic acids. King and Whitehand (1990) did not find variances on thermal resistance of *Talaromyces flavus* when the medium was supplemented with citric, tartaric, malic and lactic acids.

There is evidence that ascospore age influences the thermal resistance of microorganisms and, since it is impossible to know how long the ascospores have been in the environment, the study of the effect of ascospore age *in vitro* is relevant. An insignificant number of studies in the literature report whether this factor influences or not the growth of moulds. Slongo et al. (2005) studied thermal resistance on *N. fischeri* in pineapple juice and found the increase of ascospore ages enhanced thermal inactivation. In relation to the growth of the same microorganism, Zimmermann et al. (2011b) found that ascospore age does not influence the growth of the mould in pineapple juice.

The fruit juice industry has become a potential worldwide agribusiness market, since people are seeking healthy and highly nutritional products. There are many countries involved in the fruit juice and pulp market, mainly in the domain of tropical fruits, such as papaya and pineapple juices. According to Faylon et al. (2006), papaya, pineapple, avocado, and banana are the most common tropical fruits used in the majority of worldwide production. Papaya cultivation is favored by suitable climatic conditions, and there is a substantial commercial production of it. Papaya is regarded as a source of vitamins A and B, and as a good source of vitamin C (Telis et al., 2007).

Following the higher demand for processed foods,

researchers have increased the attention to the application of mathematical models to quantify and predict microbial responses in relation to environmental factors, ensuring the microbiological quality of food supplies and population safety (Cheroutre-Viale et al., 2006). Sant'Ana et al. (2010a) have applied the Logistic model to assess the effect of temperature and the initial number of *Byssoschlamys fulva* ascospores on the percentage of spoiled apple juice bottles. The Modified Gompertz model has been applied by Wang et al. (2010) to evaluate the ultrasonic inactivation efficacy of *Alicyclobacillus acidiphilus* and *A. acidoterrestris* in apple juice. Char et al. (2009), analyzing the response of *Listeria innocua* in orange juice affected by a combination of different levels of vanillin and mild thermal treatments has found that nonlinear semi-logarithmic survival curves were successfully fitted by using a modified version of the Gompertz model, as well as Zimmermann et al. (2011b) have modeled the growth of *N. fischeri* on pineapple juice and obtained a good fit of the Modified Gompertz model to experimental data.

Lately, papaya juice has been available for consumption in different kinds of packages like plastic bottles, laminated paperboard or glass bottles. However, there are no studies describing the growth of *B. nivea* in commercial papaya juice at a wide range of water activity and different ascospore age. Therefore, the growth of *B. nivea* on papaya juice was modeled in this research to compare it with the previous study on pineapple juice (Zimmermann et al., 2011a) and to investigate whether the different juice composition influences the effect of water activity and ascospore age on this microorganism's growth.

Materials and Methods

Mould and Ascospore Suspension

The *Byssoschlamys nivea* strain used in this research was isolated by Aragão (1989) in the Thermobacteriology Laboratory of the State University of Campinas, in Campinas, SP, and Brazil.

Firstly, cells were pre-cultivated in Potato Dextrose Agar (PDA, Himedia Laboratories, Mumbai, IND) at 30 °C for seven days. After that, the colonies were rinsed with sterile distilled water and gently scrapped from the surface. Roux bottles containing 180 mL of Malt Extract Agar (MEA, Difco Laboratories, Detroit, MI, USA) were inoculated with 0.5 mL of that pre-culture, and then these bottles were incubated at 30 °C

to reach ages of 30, 51, 60, 69 and 90 days. After each desired age, ascospore suspensions were obtained as described by Zimmermann et al. (2011a). Next, ascospores were harvested by flooding the medium surface with 25 mL sterile distilled water. The ascospore suspension was filtered through layers of gauze and centrifuged at 2000 g for 15 minutes. The suspension was washed three times with 5 mL sterile distilled water, followed by centrifugation. The absence of hyphae was confirmed through microscopy analyses. The final suspension was prepared resuspending the precipitate in a small volume of sterile water and stored under refrigeration at 4 °C for future use. Ascospore concentration was determined as described by Zimmermann et al. (2011a).

Medium

The experiments were performed on commercial acidified (acid citric) papaya juice (pH 3.53 and 12.3 °Brix) from the same brand and production lot, purchased at a local market. Sucrose and distilled water were used to adjust the values of water activity (a_w) to 0.90, 0.93, 0.95, 0.96 and 0.99; and an Aqualab (Model Series 3TE, Decagon Devices, Pullman, WA, USA) was used to verify water activity values of the adjusted medium.

Inoculation

Polyethylene terephthalate transparent bottles (PET - 250 mL) were used to conduct all the experiments. The bottles were previously sanitized with a 3 mL/L peracetic acid aqueous solution (P3 Oxônia Ativo 150, Ecolab, Barueri, SP, Brazil), for 30 minutes. The caps were also sanitized by using a 0.5 mL/L aqueous solution of the same product for the same contact time. After the contact time, bottles and caps were rinsed twice with sterile distilled water (Silva and Massaguer, 2005).

Heat treatment at 110 °C for 15 minutes was performed on papaya juice to prevent the media from contamination, followed by cooling to room temperature. The ascospores suspension was activated at 80 °C for 30 minutes, and then inoculated into the juice in the proportion of one ascospore/mL.

Mould Growth

B. nivea growth was monitored by measuring the radial growth (mm), based on the method reported by Peña et al. (2004), using a graduated ruler and a

flashlight for helping the visualization (Zimmermann et al., 2011a).

The inoculated bottles were tilted at 45 ° to increase the surface area and to facilitate the measurement of the colony. Bottles were stored at 30 °C, which is the ideal sporulation temperature of this microorganism (Tournas 1994; Pitt and Hocking, 1999); and the growth curves were analyzed until the colonies growth ceased (variable time for each condition).

Mathematical and Statistical Modeling

A typical two-step modeling approach, including primary and secondary modeling, was employed to quantify the effect of water activity and ascospore age on the kinetic parameters of *B. nivea*. Estimates of the mould growth rates were obtained by plotting changes of colony diameter against time. For each treatment, the lag phase duration (λ), the maximum specific growth rate (μ_{\max}) and the maximum diameter reached by the colony (A) were calculated by fitting the primary Modified Gompertz (Equation 01) and Logistic (Equation 02) models to the experimental data, by using the software Statistica 6.0.

$$\ln y = A \exp \left\{ -\exp \left\{ \left(\frac{\mu_{\max} \times e}{A} \right) (\lambda - t) \right\} + 1 \right\} \quad (1)$$

$$\ln y = \frac{A}{\{1 + \exp(D - Bt)\}} \quad (2)$$

The diameter of the colony is y (mm) at a given time t (h), λ is the length of lag phase (h), μ_{\max} is the maximum specific growth rate (mm/h), A is the maximum diameter reached by the colony (mm), e is a constant (2.7182); D is a dimensionless parameter and B is the relative growth in half of the time of the exponential phase (h^{-1}). D and B are used to determine the microbiological growth parameters λ and μ_{\max} (Equations 3 and 4).

$$\mu_{\max} = \frac{AB}{4} \quad (3)$$

$$\lambda = \frac{(D-2)}{B} \quad (4)$$

The influence of the statistically significant factor on mould growth parameters was described by general secondary models, the Linear model ($y = ax + b$) and the Square Root model ($\sqrt{y} = c(x - x_{\min})$), by using Excel software. The empirical parameters of the equation are a and b ; c is a constant; x corresponds to values of a_w or AA; x_{\min} corresponds the conceptual minimum a_w or AA; and y corresponds to the

parameter of interest of the primary model.

Mathematical and Statistical Comparison

The goodness of fit of the modeling approach to experimental data was evaluated by Correlation Coefficient (R^2), Mean Square Error (MSE), Bias Factor and Accuracy Factor. The Correlation Coefficient (R^2) represents the fraction of the variation that is explained. The higher the value, the better the data are predicted by the model. The Mean Square Error (MSE) is given by Equation 5 and describes the error of the model compared to the experimental data. The closer to zero, the better the fit is. The Bias Factor (Equation 6) and the Accuracy Factor (Equation 07) test the hypothesis that the model under evaluation predicts the true meaning or represents it better than another model. A Bias Factor < 1 indicates that the model is, in general, fail proof (Ross, 1996). The Bias Factor is an estimate of the average difference between observed and predicted values. The closer to 1, the better the fit is. The Accuracy Factor is the most suitable and accurate statistical parameter because it calculates the percentage of error in the prediction. This factor takes into consideration only the absolute values. The closer to 1, the lower the percentage of error is.

$$MSE = \frac{RSS}{n} = \frac{\sum (v_{observed} - v_{predicted})^2}{n - p} \quad (5)$$

$$Bias = 10^{\left(\frac{\sum \log(v_{observed} / v_{predicted})}{n}\right)} \quad (6)$$

$$Accuracy = 10^{\left(\frac{\sum |\log(v_{observed} / v_{predicted})|}{n}\right)} \quad (7)$$

The value of experimental data is given by $v_{observed}$; the value estimated by the model is given by $v_{predicted}$; n is the number of experimental observations; and p is the number of parameters of the model.

After the statistical analysis, the model that best fitted the experimental data was identified and the growth parameters obtained by this model were submitted to analysis of variance (ANOVA) with $P < 0.05$. Statistical analysis was performed by using the software Statistica 6.0.

Results

The minimum diameter to identify the colony growing of *B. nivea* in papaya juice was approximately 2 mm. According to Gibson et al. (1994), the minimum size to identify mould colonies is 3 mm, which is enough to cause rejection of the product by the consumer. The improvement in colony observation in this study may

be due to the use of a flashlight.

Representative fits of the Modified Gompertz (GM) and Logistic (L) models to the experimental data are shown in Fig. 1. Through the visual analysis of this figure, it can be noticed that both models fitted well to the experimental data and the growth curves obtained by plotting colony diameter changes over time were typical of fungal growth having a lag phase duration depending on a_w levels and ascospore ages, followed by logarithmic growth with upper asymptote to stationary phase. However, the Modified Gompertz model had a slightly superior performance on the transition between the adaptation phase and exponential growth phase. Similar behavior was also observed on the other tested conditions of this research (data not shown).

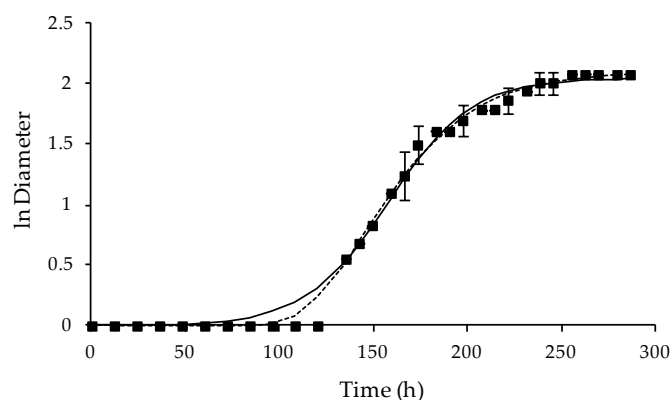


FIG. 1 GROWTH CURVE OF *B. NIVEA* IN PAPAYA JUICE FOR WATER ACTIVITY ADJUSTED TO 0.93 AND ASCOSPORE AGE TO 51 DAYS. MODIFIED GOMPERTZ MODEL (—); LOGISTIC MODEL (---); AVERAGE OF EXPERIMENTAL DATA (■); STANDARD DEVIATION (⊥). DIAMETER (MM)

Statistical parameters obtained for one situation analyzed, by fitting both models to the growth curves, can be visualized in Table 1. For all experimental data, the models showed similar performance (data not shown). It can be observed that both models showed good fit to the experimental data, since MSE was close to 0, and the Bias Factor, Accuracy Factor and Correlation Coefficient were close to 1. Although the models have shown similar statistical parameters, the Modified Gompertz model was chosen to continue this study as it had the best fit based on the analysis of R^2 (data in boldface on Table 1). The Modified Gompertz model also provided the most accurate prediction of *B. nivea* growth in pineapple juice under influence of water activity and ascospore age (Zimmermann et al. (2011a).

TABLE 1 STATISTICAL PARAMETERS OBTAINED FOR THE FIT OF MODELS OF THE MODIFIED GOMPERTZ (MG) AND LOGISTIC (L) MODELS AT THE ASCOSPORE AGE OF 51 DAYS FOR *B. NIVEA* GROWTH CURVES IN PAPAYA JUICE

Test	a_w	Model	MSE	Bias Factor	Accuracy Factor	R^2
1	0.90	MG	0.024	1.000	1.000	0.992
		L	0.024	1.000	1.001	0.990
2	0.93	MG	0.032	1.000	1.000	0.996
		L	0.036	0.998	1.002	0.995
3	0.95	MG	0.027	1.000	1.000	0.995
		L	0.026	1.000	1.003	0.993
4	0.96	MG	0.032	0.996	1.013	0.993
		L	0.025	0.995	1.004	0.990
5	0.99	MG	0.047	0.999	1.001	0.993
		L	0.055	1.002	1.002	0.991

In order to illustrate growth curves of *B. nivea* in papaya juice, only one example at each situation of ascospore age (Fig. 2) and water activity (Fig. 3) are presented. The standard deviation was not presented again in order to facilitate the visualization of the growth curves. Nevertheless, it followed a similar proportion as shown in Fig. 1.

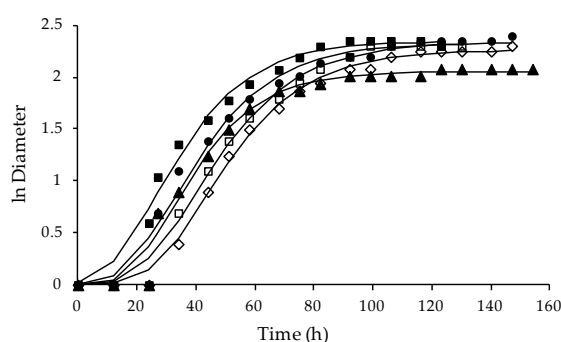


FIG. 2 GROWTH CURVES OF *B. NIVEA* IN PAPAYA JUICE FOR ASCOSPORE AGES OF 30 (\diamond), 51 (\blacktriangle), 60 (\square), 69 (\bullet) AND 90 (\blacksquare) DAYS STUDIED AT A_w OF 0.99. SYMBOLS REPRESENT THE AVERAGE OF THE DUPLICATE. MODIFIED GOMPERTZ MODEL (—). DIAMETER (MM)

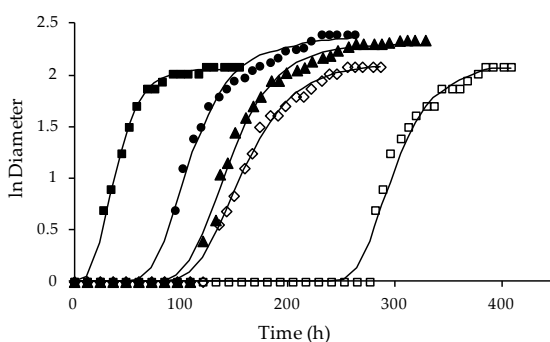


FIG. 3 GROWTH CURVES OF *B. NIVEA* IN PAPAYA JUICE FOR A_w CONDITIONS OF 0.90 (\square), 0.93 (\diamond), 0.95 (\blacktriangle), 0.96 (\bullet) AND 0.99 (\blacksquare) STUDIED AT THE ASCOSPORE AGE OF 51 DAYS. SYMBOLS REPRESENT THE AVERAGE OF THE DUPLICATE. MODIFIED GOMPERTZ MODEL (—). DIAMETER (MM)

The growth curves adjusted when a_w was 0.99 at all ascospore ages studied are shown in Fig. 2. It was possible to notice that the ascospore age showed no influence on growth parameters of *B. nivea*, since the curves were practically overlapped. Similar behavior was observed in all other studied conditions. The values of growth parameters λ , μ_{max} , and A obtained from the Modified Gompertz model for all experimental conditions analyzed for ascospore ages were submitted to the analysis of variance (ANOVA) with significance level $P < 0.05$. Ascospore age did not show a statistically significant influence on any growth parameter in the range studied (p -value > 0.138 for all parameters). Analyzing the influence of water activity and ascospore age on *B. nivea* in pineapple juice in the previous research (Zimmermann et al., 2011a), it was found that ascospore age did not influence mould growth. Comparing the results on both studies, it was possible to report that ascospore age did not influence *B. nivea* growth, even in different juice compositions such as pineapple and papaya juices, in the range studied.

Throughout Fig. 3, when the ascospore age was 51 days at all studied water activity levels, it can be observed that the decrease of water activity caused an increase in the lag phase duration and also a decrease in the specific growth rate, mainly for the studied minimum water activity (0.90), since high sugar concentration was necessary to reach the desired a_w . The fast growth observed on the highest values of water activity allows the visual identification of the contaminant mould colony in a very short time when compared to 240-day shelf life of commercial juice. At the present study, when a_w varied from the lowest to the highest condition, the mould colony was identified in approximately 17 and 266 hours of incubation at 30 °C, respectively. Statistically, water activity had a significant influence on the growth parameters λ and μ_{max} , giving a p -value equal to 0.000 for both cases, while it did not show a significant influence on the parameter A (p -value = 0.114), in the studied range. The importance of the lag phase duration (λ) and the maximum specific growth rate (μ_{max}) for the juice industry is related to the identification of the microorganism colony by consumers. Once the adaptation phase is completed, microorganism growth can hardly be controlled. The longer the adaptation phase and the lower the maximum specific growth rate, the better it is for product shelf life. On the previous study (Zimmermann et al., 2011a), it was also found that water activity influenced *B. nivea* growth in

pineapple juice. When the water activity was changed from 0.90 to 0.99, the lag phase duration decreased and the specific growth rate increased. The mould colony was identified in approximately 51 and 285 hours of incubation at 30 °C, respectively, when the water activity was adjusted from the lowest to the highest condition, considering the average of all analyzed ascospore ages.

Since a_w was statistically significant to the growth parameters λ and μ_{\max} , secondary mathematical models were applied using the data previously obtained by primary modeling of the experimental data. Fig. 4 and 5 present the average of studied ages at 30, 51, 60, 69 and 90 days and the standard deviation of λ and μ_{\max} obtained in each a_w condition, respectively. The mathematical relation between a_w and λ obtained by fitting of the Linear model is given by Equation 8. The model showed a very good prediction of the experimental data, since the correlation coefficient (R^2) obtained was 0.994.

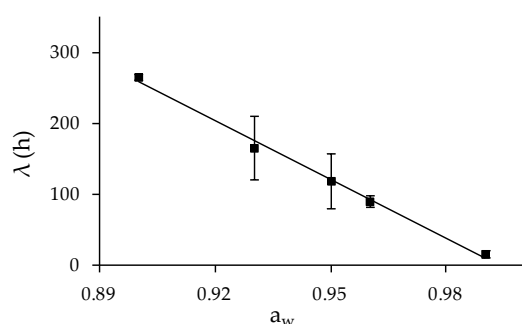


FIG. 4 RELATION OF λ AND A_w DURING THE GROWTH OF *B. NIVEA* IN PAPAYA JUICE, OBTAINED BY FITTING THE SECONDARY LINEAR MODEL. AVERAGE OF λ (■); LINEAR MODEL (—); STANDARD DEVIATION (⊥)

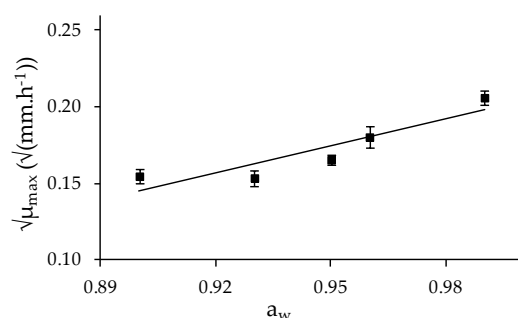


FIG. 5 RELATION OF μ_{\max} AND A_w DURING THE GROWTH OF *B. NIVEA* IN PAPAYA JUICE, OBTAINED BY FITTING THE SECONDARY SQUARE ROOT MODEL. AVERAGE OF μ_{\max} (■); SQUARE ROOT MODEL (—); STANDARD DEVIATION (⊥)

$$\lambda = -2,744a_w + 2,728 \quad (8)$$

The relation between a_w and μ_{\max} can be described by Equation 9. The Square Root model exhibited a

reasonably good fit to the experimental data in terms of correlation coefficient (R^2), which obtained a value of 0.831.

$$\sqrt{\mu_{\max}} = 0.592(a_w - 0.388) \quad (9)$$

Discussion

The results of the current study confirmed the results of the previous research (Zimmermann et al., 2011a). By comparing the results, it was possible to conclude that even using different fruit juices, water activity of the growth media influenced growth parameters of *B. nivea*; and ascospore age did not influence growth parameters of the mould, at least up to 90 days of the incubation. Every acid fruit juice has different characteristics, such as nutritional composition. However, the growth parameters of *B. nivea* at the same water activity level were not influenced by the kind of fruit juice, since the parameter values were very close to each other. It is important to note that inoculation of the studied microorganism was made directly in the fruit juices and the observation was performed in PET transparent bottle, which was possible to observe the growth of the mould.

Panagou et al. (2010), studying *B. nivea* growth at 30 °C on a synthetic medium as a function of a_w (0.88 to 0.99), found that λ varied from 1.40 to 10.56 days when a_w went from 0.99 to 0.90, respectively, and μ_{\max} varied from 23.35 to 0.84 mm/day when a_w went from 0.99 to 0.90, respectively. Bouras et al. (2009), studying *Pyrenophora tritici-repentis* growth in a wheat-based medium, reported that mould growth greatly decreased when a_w was reduced from 0.99 to 0.95, even under the most favorable temperature condition (25 °C) for this microorganism. The results found in this research are in accordance to the data cited above, since the fast mould growth was observed when water activity was at the maximum level studied (0.99), and growth decreased drastically when water activity was at the minimum level studied (0.90) (Fig. 3). In addition, the influence of water activity on the growth parameters λ and μ_{\max} of *B. nivea* in papaya juice was confirmed by statistical analyses.

A number of studies can be found in the literature reporting the influence of ascospore age on thermal inactivation of microorganisms. Dijksterhuis and Teunissen (2004) showed that ascospore age affects high pressure inactivation. Slongo and Aragão (2006) found that ascospore age influenced the heat resistance of *N. fischeri* in papaya and pineapple juices.

Chapman et al. (2007) also reported that older ascospores of *B. fulva*, *B. nivea*, *N. fischeri* and *N. spinosa* were more resistant to high pressure than younger ascospores. However, it was challenging to find appropriate literature data that evaluate whether ascospore age influences mould growth. Araújo and Rodrigues (2004) reported that young spores of *Aspergillus fumigatus* and *A. niger* showed higher germination rates than old spores; however, spore age did not affect the germination of *A. flavus*. According to Dantigny and Nanguy (2009), there was a tendency of an increase in the germination time as the spore age was increased, although this behavior was not observed in this study. Differences in response among the different species and among different strains of a single species were found at the literature. However, it was evidenced that ascospore age may influence more the inactivation of moulds than their growth.

The growth curves obtained in this study almost overlap indicating that ascospore age did not affect mould growth in the studied range. The statistical analyses confirmed no statistically significant influence ($p > 0.05$) of ascospore age on any growth parameter of *B. nivea* in papaya juice; the same result was found in the previous study using pineapple juice as growth media as well.

An additional point that could be noticed on the comparison of the studies is the way the colonies of *B. nivea* grew in the different fruit juices. In papaya juice, colony growth could be observed in the middle of the bottle juice. However, the same situation was not observed for pineapple juice, since this juice separated into two phases. Then, the mould colony growth could only be observed on the surface of the pineapple juice. Therefore, the colonies of *B. nivea* took longer to be seen on pineapple juice than papaya juice took. For example, when the water activity was adjusted to 0.99, the first colony growth was identified in papaya juice about 17 hours after inoculation of the mould. However, about 51 hours was the necessary to identify the first colony growth after inoculation of *B. nivea*, using pineapple juice as growth media.

Conclusion

The results of this study suggested models to describe the influence of water activity on *B. nivea* growth in fruit juices. These models can be applied in the range of studied water activity, irrespective of the ascospore age, since it was not significant as well as the kind of fruit juice at least pineapple and papaya juices. The

results also suggested that by decreasing water activity of fruit juices, the industry can extend the shelf life of these products and/or guarantee their quality and safety; since the time for moulds to develop on lower water activity juices was significantly longer than in juices that had higher water activity.

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